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(54) Title: PHOSPHINIC CREATINE COMPOUNDS HAVING ANTIVIRAL ACTIVITY

(57) Abstract

Novel phosphinic creatine compounds are described. A method for treating a subject for a viral infection by administering an antiviral effective amount of a phosphinic creatine compound to a subject also is described. The compounds can be administered as therapeutic compositions which contain an antiviral effective amount of a phosphinic creatine compound and a pharmaceutically acceptable carrier.

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PHOSPHINIC CREATINE COMPOUNDS HAVING ANTIVIRAL ACTIVITY

Background Of The Invention

A number of antiviral agents are now available for clinical use. Some antiviral agents, such as trifluridine, ribavarin (virazole), and interferon have a wide spectrum of target viruses. Goodman Gilman, A. et al. eds. The Pharmacological Basis of Therapeutics (Pergamon Press, New York 1990) 1182-1201. Trifluridine (5-trifluoromethyl-2'-deoxyuridine) is a fluorinated pyrimidine deoxynucleoside that inhibits viral DNA synthesis. Trifluridine is active against herpes simplex virus, cytomegalovirus, vaccinia virus, and some strains of adenovirus. Ribavirin, which is an analogue of the purine precursor 5'-aminoimidazole-4-carboxamide, exhibits antiviral activity against a range of viruses, including both RNA and DNA-containing viruses. In addition, most RNA and DNA viruses are sensitive to the antiviral activity of interferons, which are glycoproteins that can bind to specific cell-surface receptors and inhibit viral penetration or uncoating, synthesis or methylation of mRNA, translation of viral proteins, or viral assembly and release.

Other antiviral agents are specific for particular viruses. Examples of such viruses include anti-herpes virus agents such as acyclovir, a synthetic purine nucleoside analogue (9-[(2-hydroxy-ethoxy)methyl]guanine), and ganciclovir, a more toxic relative of acyclovir which is also active against cytomegalovirus. Goodman Gilman, A. et al. eds. The Pharmacological Basis of Therapeutics (Pergamon Press, New York 1990) 1182-1201. Because of its toxicity, ganciclovir is generally reserved for sight- or life-threatening infections with cytomegalovirus. Another less potent anti-herpes virus agent is vidarabine (adenine arabinoside, ara-A), which is an analogue of adenosine. Goodman Gilman, A. et al. eds. The Pharmacological Basis of Therapeutics (Pergamon Press, New York 1990) 1182-1201. Other anti-herpes virus agents include phosphonoformic acid (PFA, foscarnet), and phosphonoacetic acid (PAA). PFA and PAA are potent and highly specific inhibitors of herpes virus DNA polymerases. And despite the drawbacks to its use, e.g. it is nephrotoxic and mutants resistant to it emerge rapidly, PFA is now undergoing clinical trials in immunosuppressed patients infected with cytomegalovirus. Other analogues of ribonucleosides and deoxyribonucleosides which strongly inhibit herpes virus DNA polymerases but not cellular DNA polymerases, possess low cytotoxicity, and are undergoing clinical trials. Among them are analogues of thymidine, such as (E)-5-(2-bromovinyl)-2'-deoxyuridine (BVdU), and analogues of cytosine, such as 1-(2'-deoxy-2'-F-β-D-arabinofuranosyl)-5-iodocytosine (FIAC).

Of particular interest in the past decade have been anti-human immunodeficiency virus (HIV) agents such as zidovudine (azidothymidine, also known as retrovir or AZT) and the dideoxynucleosides, dideoxythymidine (ddA), dideoxycytosine (ddC), and dideoxyinosine (ddI). Katzung, B.G. ed. Basic and Clinical Pharmacology (Appleton & Lange, Connecticut 1992). Zidovudine is an inhibitor of retrovirus reverse transcriptases. As

an important aspect of its antiviral activity, zidovudine, as a thymidine analogue, causes chain termination during DNA synthesis. Other anti-HIV agents which are synthetic dideoxynucleosides and that act on viral DNA polymerase (reverse transcriptase) so that synthesis is inhibited and virus replication is markedly decreased are ddA, ddC, and ddI. Recently, the methyl phosphonate derivative 9-(2-phosphonylmethoxy-ethyl)adenine (PMEA) has been found to be a strong inhibitor of retrovirus multiplication and a selective inhibitor of HIV replication in human T-lymphocytes. Indeed, the anti-HIV activity of PMEA exceeds that of zidovudine.

In contrast to most other infectious agents, viral replication depends primarily on the metabolic processes of the invaded cell. Goodman Gilman, A. et al. eds. The Pharmacological Basis of Therapeutics (Pergamon Press, New York 1990) 1182-1201. Thus, agents which inhibit or cause the death of viruses are also likely to injure the invaded host cells. For this reason, the most useful antiviral agents are those that inhibit processes specific to a given virus, such as attachment, uncoating, replication, or virus-directed macromolecular synthesis. Goodman Gilman, A. et al. eds. The Pharmacological Basis of Therapeutics (Pergamon Press, New York 1990) 1182-1201. The demand for the development of such antiviral agents has increased with the rapidly increasing incidence of virally-based diseases such as cytomegalovirus infections in immunocompromised patients, recurrent genital herpes simplex virus infections, and most importantly, acquired immunodeficiency syndrome (AIDS).

Summary Of The Invention

The present invention is based, at least in part, on the discovery of novel phosphinic creatine compounds. The phosphinic creatine compounds of the present invention have an anti-viral effect and are more soluble in pharmaceutically acceptable carriers than their corresponding non-phosphinic creatine compound counterparts. The phosphinic creatine compounds can be used to treat a subject for viral infection and/or for reducing or eliminating the spreading of virus from cell to cell. The method of the present invention involves administering an antiviral effective amount of a phosphinic creatine compound to a subject such that the subject is treated for viral infection. The treatment can be of a preexisting viral infection or the treatment can be a prophylactic treatment in that it can prevent the occurrence of a viral infection within the subject.

The present invention even further pertains to the novel phosphinic creatine compounds and therapeutic compositions containing the compounds for treating a subject for a viral infection. The therapeutic compositions contain an antiviral effective amount of a phosphinic creatine compound and a pharmaceutically acceptable carrier. Another aspect of this invention is a packaged antiviral agent being a phosphinic creatine compound packaged with instructions for use of the phosphinic creatine compound as an antiviral agent.

Brief Description Of The Drawings

Figure 1 is a graph depicting the inhibition of human cytomegalovirus (HCMV) plaque formation on human embryonic lung fibroblast cells by a phosphinic creatine compound.

Figure 2 is a graph depicting the inhibition of herpes simplex virus-1 (HSV-1) plaque formation on human embryonic lung fibroblast cells by a phosphinic creatine compound.

Detailed Description Of The Invention

The present invention provides a method for treating a subject for viral infection. The method involves administering an antiviral effective amount of a phosphinic creatine compound to a subject being treated for viral infection such that the subject is treated for viral infection.

The term "subject" is intended to include subjects susceptible to viral infection. The subject can have or be susceptible to a viral infection at the time of treatment. Examples of subjects include humans, dogs, cats, pigs, cows, horses, rats, rabbits, and mice.

The language "viral infection" is intended to encompass infections caused by a virus. The virus can be a DNA or an RNA virus. Examples of types of DNA viruses intended to be encompassed by the present invention include herpes viruses, respiratory syncitial virus (RSV) and adenoviruses. Examples of types of RNA viruses include influenza and human immunodeficiency virus (HIV). Examples of herpes viruses include herpes simplex virus type 1 (HSV-1), herpes simplex virus type 2 (HSV-2), cytomegalovirus (CMV), (e.g., human cytomegalovirus (HCMV), varicella zoster virus (VZV), Epstein Barr virus (EBV), human herpes virus type 6 (HHV-6), and human lymphotrophic virus type VI (HLV-VI). Examples of adenoviruses include adenovirus 5 and adenovirus 2.

The term "administering" is intended to include routes of administration which allow the phosphinic creatine compound to perform its intended function of providing treatment for or protection against viral infection. Examples of routes of administration which can be used include injection (subcutaneous, intravenous, parenterally, intraperitoneally, etc.), oral, inhalation, transdermal, and rectal. The injection can be bolus injections or can be continuous infusion. Depending on the route of administration, the phosphinic creatine compound can be coated with or in a material to protect it from natural conditions which may detrimentally effect its ability to perform its intended function. The phosphinic creatine compound can be administered alone or further can be co-administered with a pharmaceutically acceptable carrier. Further, the compounds can be administered as a mixture of phosphinic creatine compounds which also can be in a pharmaceutically acceptable carrier. The phosphinic creatine compounds even further can be coadministered with other different reagents useful for treating viral infection, e.g., other creatine compounds or art-recognized anti-viral agents.

The phosphinic creatine compounds can be coadministered with at least one other anti-viral agent (for combination therapy purposes). There are many art-recognized antiviral agents presently available as described in the "Background of the Invention" section which is reiterated here. The antiviral agent can be an agent having an adverse effect on viral infection as described below for the phosphinic creatine compounds of the present invention. The agent can be a material which inhibits the polymerase thereby preventing replication of the viral genome, e.g., foscarnet. The agent further can be a material which becomes incorporated within or binds to the viral genome preventing replication. An example of a type of antiviral agent is a nucleoside. Examples of nucleosides include acyclovir, ganciclovir, idoxoridine, trifluoridine, vidarabine, dideoxyinosine, and azidothymidine. Other antiviral agents include antisense molecules or immunoglobulins directed against the virus.

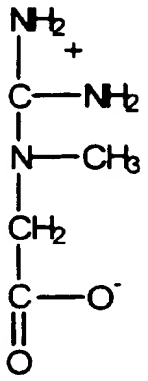
The language "treated for viral infection" is intended to include treatments which result in the reduction or elimination of a symptom(s) or condition(s) associated with a preexisting viral infection or treatments which prevent the occurrence of a viral infection or significantly reduce the occurrence of a symptom(s) or condition(s) associated with a viral infection within the subject. Therefore, the present invention provides a prophylactic means of preventing viral infection from ever occurring.

The symptoms or conditions associated with a viral infection can vary depending on such factors as the particular virus, the immune state of the subject (e.g. immunocompromised subjects can have symptoms which vary from their non-immunocompromised counterparts), and the severity of the infection. Examples of symptoms or conditions associated with viral infection include tissue damage, inflammation, edema, interruption of visual acuity (CMV retinitis), elevated body temperature, pneumonia-like symptoms, and ischemia. Examples of conditions or diseases associated with viral infection which are intended to be part of this invention include retinitis, encephalitis, enteritis, interstitial, pneumonia, and hepatitis. Specific examples include cytomegalovirus chorioretinitis (CMV retinitis), e.g. HCMV retinitis, and herpes simplex virus meningitis (HSV meningitis).

Cytomegalovirus (CMV) chorioretinitis (retinitis) is a disease or condition of the retina of the eye caused by infection with CMV. HCMV retinitis is caused by HCMV. This disease or condition is prevalent in immunocompromised hosts, e.g. hosts suffering from AIDS, newborns, or transplant recipients.

The language "an antiviral effective amount of a phosphinic creatine compound" in the context of this invention is that amount necessary or sufficient to significantly reduce or eliminate a symptom(s) or condition(s) associated with a viral infection or necessary or sufficient to prevent the occurence of a symptom(s) or condition(s) associated with a viral infection. This amount can vary depending on such factors as, particular virus or condition, the weight of the subject and severity of the condition or symptom.

Creatine (also known as N-(amidinomethyl-N-methylglycine, methylglycosyamine or N-methyl-guanidino acetic acid) is a well-known substance (see The Merck Index Ninth Edition, No. 2556 (1976)) and its formula is as follows:



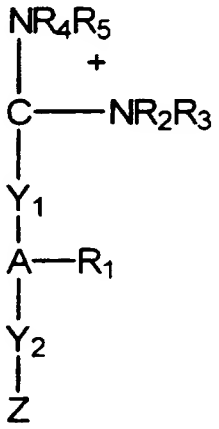
Creatine and phosphorylated creatine are generally present in the muscular tissue, brain and other organs of many vertebrates and the naturally occurring product that is commercially available is typically extracted from meat. Creatine can be chemically synthesized using conventional techniques such as by heating cyanamide with sarcosine (Strecher Jahresber. Chem. (1868), 686; cf. Volhard Z. Chem. 5,318 (1869); Paulmann, Arch. Pharm. 232, 638 (1894); Bergmann et al. Z. Physiol. Chem. 173, 80 (1928); and King J. Chem. Soc. (1930), 2374).

The language "phosphinic creatine compound" is intended to include phosphinic creatine and compounds which are structurally similar to phosphinic creatine (e.g., phosphinic creatine analogs) which contain a phosphinic moiety. The phosphinic creatine compounds of this invention all contain a phosphinic moiety (replacing the carboxylic acid or carboxylate moiety of its corresponding creatine compound counterpart (e.g., see creatine compounds described in commonly owned copending applications USSN 08/185,438 filed on January 21, 1994 and USSN 08/204,995 filed on March 2, 1994). The phosphinic moiety can be a phosphinic acid moiety or other moieties encompassed by the definition for "Z" set forth in formula I below. Phosphinic creatine was known prior to the present invention. All other phosphinic creatine compounds described herein are novel and are part of the present invention. The phosphinic creatine compounds of this invention can be used to treat a subject for viral infection, as described herein, or can be used for other purposes as previously described for their corresponding non-phosphinic creatine compound counterparts (see, e.g., the utilities described in the copending applications or issued patents cited below) The language "phosphinic creatine compound" also is intended to include pharmaceutically acceptable salts of the compound. The language "phosphinic creatine compound" can also include "mimics" or "inhibitors of creatine kinase" which contain a phosphinic moiety. "Mimics" is intended to include compounds which may not be structurally similar to creatine

but mimic the therapeutic activity of creatine or structurally similar creatine compounds. The "inhibitors of creatine kinase" are compounds which inhibit the activity of the enzyme.

The phosphinic compounds of the present invention are more soluble than their corresponding non-phosphinic creatine compound counterparts. The phosphinic compounds preferably are five times more soluble than their corresponding non-phosphinic creatine compound counterparts, more preferably ten times more soluble, and most preferably at least twenty times more soluble using the assay described in Example 4 below.

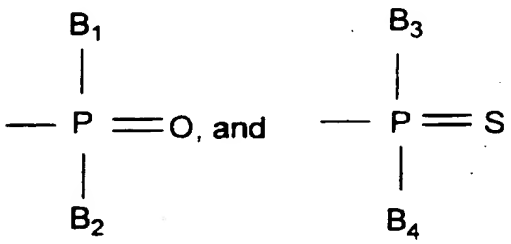
The phosphinic creatine compounds can be modified versions of the creatine compounds or analogs previously described in other copending applications or issued U.S. patents. Creatine compounds have previously been described in copending application Serial No. 08/204,995, entitled *The Use of Creatine Compounds for Treating Cachexia* filed on March 2, 1994; copending application Serial No. 08/185,438, entitled *Methods of Inhibiting Undesirable Cell Growth Using A Combination of A Creatine Compound and A Hyperplastic Inhibitory Agent* filed January 21, 1994; copending application Serial No. 07/061,677 entitled *Methods of Treating Body Parts Susceptible to Ischemia Using Creatine Analogs*, filed May 14, 1993; copending application Serial No. 08/009,638 entitled *Creatine Phosphate, Creatine Phosphate Analogs and Uses Therefor*, filed on January 27, 1993; and copending application Serial No. 07/812,561 entitled *Creatine Analogs Having Antiviral Activity*, filed December 20, 1991; and copending application Serial No. 07/610,418 entitled *Method of Inhibiting Transformation of Cells in Which Purine Metabolic Enzyme Activity is Elevated* filed on November 7, 1990. The entire contents of each of the copending applications are herein expressly incorporated by reference, along with their published foreign counterparts; and all of the creatine compounds along with their methods of synthesis and selection discussed in the aforementioned applications are intended to be part of this invention unless specifically stated otherwise. The preferred phosphinic creatine compounds of this invention are those encompassed by formula (I) set forth below:



(I)

wherein A is selected from the group consisting of N or CH:

Z is selected from the group consisting of



wherein B_1-B_4 are each independently selected from hydrogen and OX4;

X_1-X_4 are each independently selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl and pharmaceutically acceptable salts;

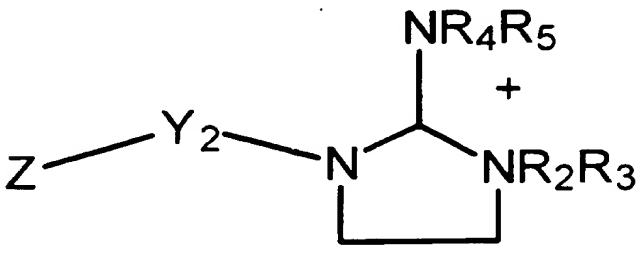
Y_1 and Y_2 are each independently selected from the group consisting of a direct bond, alkylene, alkenylene, alkynylene and alkoxylene;

R_1 is selected from the group consisting of hydrogen, hydroxyl, alkyl, alkenyl, alkynyl, and alkoxyl; and

R_2 - R_5, if present, are each independently selected from the group consisting of hydrogen, a phosphorus containing moiety, alkyl, alkenyl, alkynyl, alkoxy and haloalkyl,

wherein A may form a ring structure with one of the nitrogens in the amidino moiety or with Y_2.

A preferred subgenus of the above formula (I) are compounds encompassed by formula (II) set forth below wherein the variables are as defined above in formula (I).



(II)

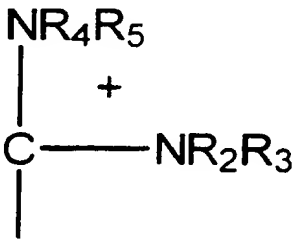
The alkylene, alkenylene, alkynylene, alkyl, alkenyl and alkynyl groups (hereinafter hydrocarbon groups) may have straight or branched chains. The unsaturated groups may have a single site of unsaturation or a plurality of sites of unsaturation. The hydrocarbon groups preferably have up to about ten carbons, more preferably up to about six carbons, and most preferably up to about three carbons. A hydrocarbon group having three carbon atoms

or less is considered to be a lower hydrocarbon group. For example, an alkyl group having three carbon atoms or less is a lower alkyl. Examples of lower hydrocarbon groups which may be used in the present invention include methyl, methylene, ethyl, ethylene, ethenyl, ethenylene, ethynl, ethynylene, propyl, propylene, propenyl, propenylene, propynyl, and propynylene. Examples of higher hydrocarbon groups (from four to about ten carbons) include butyl, t-butyl, butylene, butenyl, butenylene, and butynyl, butynylene, nonyl, nonylene, nonenyl, nonenylene, nonynyl, and nonynylene.

The alkoxy, haloalkyl, alkoxyene, and haloalkylene groups (hereinafter substituted hydrocarbon groups) are alkyl or alkylene groups substituted with one or more oxygen or halogen atoms. The alkoxy and haloalkyl groups also may be straight or branched chain and preferably are made up of up to about ten atoms (including carbon, oxygen or halogen), preferably up to about six atoms, and most preferably up to about three atoms. The term halogen is art-recognized and includes chlorine, fluorine, bromine, and iodine. Examples of substituted hydrocarbon groups which are useful within this invention are similar to the examples of the hydrocarbon groups set forth above except for the incorporation of oxygen(s) or halogen(s) into the groups.

The term pharmaceutically acceptable salt is intended to include pharmaceutically acceptable salts capable of being solvated under physiological conditions. Examples of such salts include sodium, e.g. disodium, potassium, e.g. dipotassium, and hemisulfate. The term further is intended to include lower hydrocarbon groups capable of being solvated under physiological conditions, i.e. alkyl esters, e.g. methyl, ethyl and propyl esters.

For purposes of this invention, the amidino moiety of formula I is depicted below:



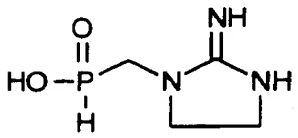
The nitrogens in this moiety can form a ring structure with A or with X_2. The ring can be a hydrocarbon ring or a hetero ring containing atoms such as O, N or S. The ring structure further can be a single ring or alternatively can be a fused ring system. The preferred ring structures are single rings having five, six or seven ring members and most prefereably five membered rings such as those present in cyclocreatine- or carbocreatine-like compounds.

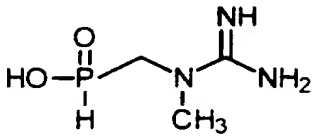
The phosphinic creatine compounds of this invention preferably possess inherent characteristics enhancing their ability to perform their intended function of preventing viral infection. For example, the phosphinic creatine compounds preferably have a

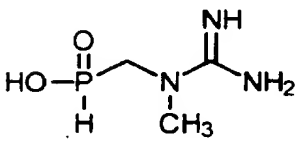
solubility which allows them to be delivered in a pharmaceutically acceptable formulation. A saturated solution is not considered to be a pharmaceutically acceptable formulation. The phosphinic creatine compounds further can be selected based on their ability to act as a substrate for creatine kinase. Some of the phosphinic creatine compounds which are useful in this invention are listed in Table 1 below

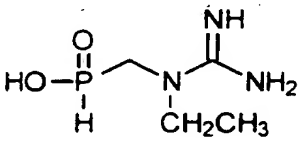
TABLE 1

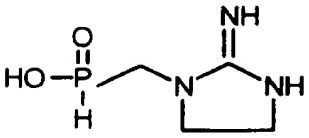
Prephosphagens

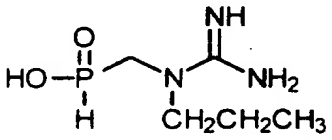


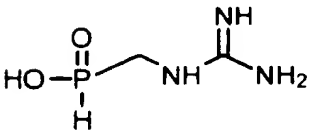


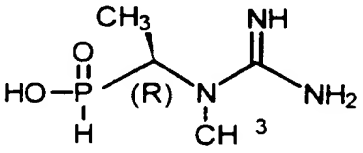


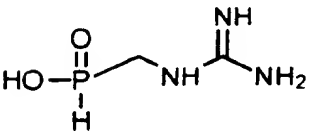


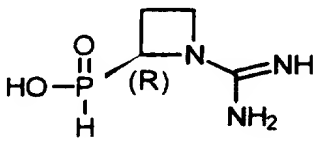


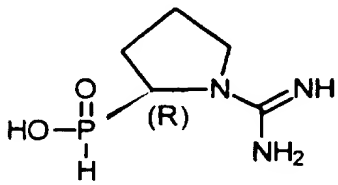




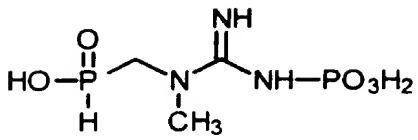


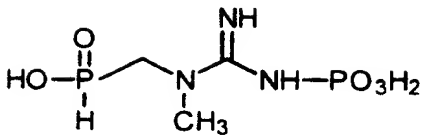


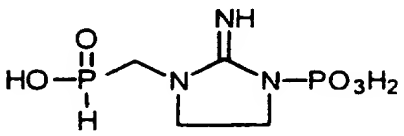


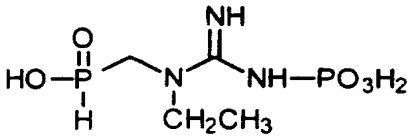


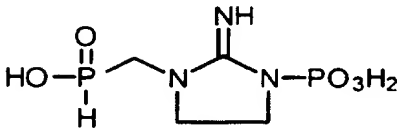
Phosphagens

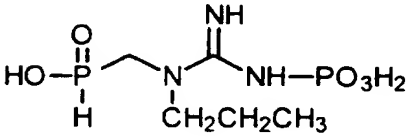


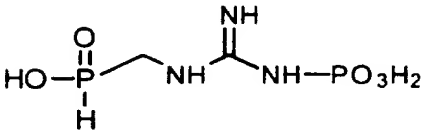


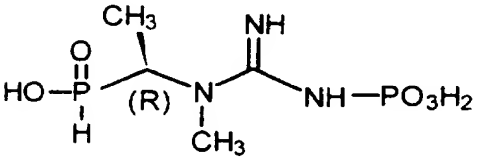


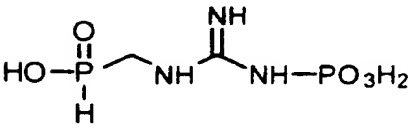


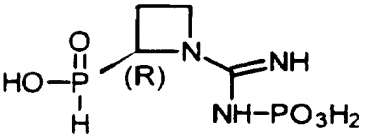


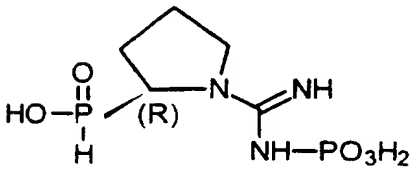












The phosphinic creatine compounds can be synthezized as described in Example 1 below . It should be understood that the ordinarily skilled artisan would know to make modifications to the synthesis method of Example 1 for the purpose of making a different phosphinic creatine compound. For example, a different starting material can be used. Even further, creatine can be used as the starting material for synthesizing at least some of the compounds encompassed by formula I. Appropriate synthesis reagents, e.g. alkylating, alkenylating or alkynylating agents can be used to attach the respective groups to target sites, e.g. a nitrogen in the guanidino moiety. Appropriate protecting groups can be employed to prevent reaction at undesired sites in the molecules. If the phosphinic creatine compound contains a ring structure, i.e. one of the nitrogens in the amidino moiety forms a ring with "A" or "Y_2", then the compound can be synthesized in a manner analogous to that described

for cyclocreatine (Wang, T., J. Org. Chem. 39:3591-3594 (1974)). The various "R", "X" groups can be introduced before or after the ring is formed and "Y" group can be introduced before the ring is formed. Phosphinic creatine compounds having the phosphinic moiety attached to an alpha carbon can be made in a manner analogous to that described in Example 1 below. Phosphinic creatine compounds having the phosphinic moiety attached to a beta carbon can be made by introducing a halogen group which subsequently is involved in the phosphinilation step. Further, the phosphinic creatine compounds can be made in an analogous manner to their corresponding phophonic counterparts.

Many creatine compounds have been previously synthesized and described (Rowley et al., J. Am. Chem. Soc. 93:5542-5551 (1971); McLaughlin et al., J. Biol. Chem. 247:4382-4388 (1972); Nguyen, A.C.K., "Synthesis and enzyme studies using creatine analogs", Thesis, Dept. of Pharmaceutical Chemistry, Univ. Calif., San Francisco (1983); Lowe et al., J. Biol. Chem. 225:3944-3951 (1980); Roberts et al., J. Biol. Chem. 260:13502-13508 (1985); Roberts et al., Arch. Biochem. Biophys. 220:563-571 (1983); and Griffiths et al., J. Biol. Chem. 251:2049-2054 (1976)). The phosphinic creatine compounds of this invention can be synthesized chemically or enzymatically. The chemical conversion of the prephosphagens (see Table 1) to the respective phosphagens can be done in the same manner as that descibed by Annesley et al. (Biochem. Biophys. Res. Commun. (1977) 74:185-190). Disodium salts, e.g. disodium salts, of the phosphinc creatine compounds can be prepared as described in aforementioned copending application Serial No. 08/009,638 filed on January 27, 1993. The contents of all of the aforementioned references are expressly incorporated by reference. Further to the aforementioned references, Kaddurah-Daouk et al. (WO92/08456) also provide citations for the synthesis of a plurality of creatine compounds (see Examples 2 and 3 including Table 4). The contents of the entire Kaddurah-Daouk et al. published patent application including the contents of any references cited therein also are expressly incorporated by reference.

Some specific examples of phosphinic creatine compounds of the present invention include phosphinic cyclocreatine (PhCCr), phosphinic CCr phosphate, phosphinic creatine, phosphinic creatine phosphate, and phosphinic homocyclocreatine.

The present invention further pertains to a therapeutic composition for treating a subject for viral infection. The therapeutic composition contains an antiviral effective amount of a phosphinic creatine compound and a pharmaceutically acceptable carrier. The language "antiviral effective amount" and "phosphinic creatine compound" are as defined above.

The language "pharmaceutically acceptable carrier" is intended to include substances capable of being coadministered with the phosphinic creatine compound(s) and which allow the compound to perform its intended function of treating a viral infection. Examples of such carriers include solutions, solvents, dispersion media, delay agents, emulsions and the like. The use of such media and agents for pharmaceutically active substances is well known in

the art. Any conventional media and agent compatible with the phosphinic creatine analog can be used within this invention.

The present invention even further pertains to packaged antiviral agents containing a phosphinic creatine compound as defined above packaged with instructions for using the phosphinic creatine analog as an antiviral agent. The instructions would provide such information as the appropriate dose of the phosphinic creatine compound for treating a particular virus.

The following invention is further illustrated by the following examples which should in no way be construed as being further limiting. The contents of all references, pending patent applications and published patent applications, cited throughout this application are hereby incorporated by reference. It should be understood that the *in vitro* assays used in the examples are accepted in that a demonstration of efficiacy in these assays is predictive of efficiacy in humans.

EXAMPLES

Example 1- Synthesis of a Phosphinic Creatine Compound

Methods and Materials

General

Reagent-grade chemicals were used as purchased from Aldrich Chemical Company, Inc. unless otherwise stated. Absolute alcohol was purchased from Commercial Alcohols; other solvents were purchased from Caledon Laboratories. Chloromethylphosphinic acid was prepared using the route described by E. Uhing, et al.(*J. Am. Chem. Soc.* **1961,** 83, 2299-303). The precursor chloromethylphosphonic dichloride was synthesized by the method of von Schwarzenbach, et al.(*Helv. Chim. Acta* **1949,** 32, 1175). HPLC traces were obtained using a reverse-phase Regis C_{18} fully endcapped 25 cm x 4.6 mm ID column with spherical 5 micron (OD) particles (VAL-U-PAK HP, Regis product # 731901), 0.03 M K_2HPO_4 (pH 7.5) eluent with a flow rate of 0.5 mL/min, and U.V. detection at 210 or 215 nm. ^{1}HNMR spectra were obtained on a Bruker 400 MHz or a Varian Gemini 300 MHz spectrometer. Chemical shifts were measured relative to partially deuterated solvent peaks, but were reported relative to tetramethylsilane. In all cases high frequency chemical shifts are reported as positive. Elemental analyses were performed by Canadian Microanalytical Service Ltd., Delta, B.C., Canada.

Preparation of N-(2-aminoethyl)aminomethylphosphinic acid

To a 12-liter round bottom flask, fitted with an overhead mechanical stirrer and a 500 mL addition funnel were added 7.0 L of ethylenediamine (104.7 mol) and 2.6 kg of ice (i.e. approximately 2.6 L of water). With stirring, the flask was cooled on an ice bath until the temperature of the ethylenediamine solution reached 30° C. 180.0 g of chloromethylphosphinic acid (1.57 mol) and 400 mL of water were then transferred to the

addition funnel and this solution was added dropwise to the reaction flask with stirring. The reaction solution was stirred overnight, and then concentrated (over a three day period) in a rotary evaporator. The dirty-yellow residue obtained was extracted with absolute ethanol (2.55 L in total) in three portions with the insoluble material (34.0 g of undesired side product) removed by suction filtration. Solvent was removed from the red-orange filtrate in a rotary evaporator with the aid of a 60°C water bath. Residual water and ethylenediamine were removed using a rotary evaporator attached (in series) to a trap cooled on dry ice, a trap cooled on dry ice/acetone, and a vacuum pump. This resulted in 386.7 g of a reddish-caramel colored viscous liquid which contained the intermediate N-(2-aminoethyl)-aminomethylphosphinic acid and residual ethylenediamine.

Preparation of 2-Iminoimidazolidine-1-methylphosphinic acid (phosphinic cyclocreatine)

The crude N-(2-aminoethyl)aminomethylphosphinic acid was transferred, with the aid of 200 mL of water, to a 50 liter round bottom flask, which contained a teflon coated stirring bar and was equipped with a thermometer and a 500 mL addition funnel. Sodium hydroxide pellets (140.60g, 3.00 mol) and 240 mL of water were added to the reaction flask and the mixture was cooled with stirring to a temperature of 0°C using an isopropanol/dry ice bath. 322.7 g (3.05 mol) of cyanogen bromide dissolved in approximately 350 mL of methanol was then transferred to the addition funnel. The cyanogen bromide solution was added dropwise to the chilled reaction mixture with efficient stirring while the temperature of the reaction solution during the addition period was kept between 5 and 10°C. The addition funnel was then rinsed with a total of 140 mL of methanol, and the washings were added to the reaction flask. The solution was stirred overnight, and the methanol solvent was removed on a rotary evaporator. Residual water was then removed using a rotary evaporator attached (in series) to a trap cooled on dry ice, a trap cooled on dry ice/acetone, and a vacuum pump to get a thick ochre yellow product. This crude product was extracted with 3.2 L of boiling methanol and suction filtered to remove sodium halide salts which were washed with 100 mL of hot methanol. 1.4 L of diethyl ether was added to the amber-red filtrate, and the mixture was allowed to sit overnight. An additional 700 mL of ether was then added to precipitate more solid which was suction filtered, washed with 200 mL of ether and air dried. This crude material (86.1 g), 1 L of methanol and 100 mL of water were brought to a boil with stirring, and the fine white solid present (13.0 g) was suction filtered and dried over KOH *in vacuo*. A clumpy pale yellow solid (1.5 g) was left behind in the washing flask. The yellow solid was stirred with 85 mL of boiling 90% methanol and transferred to a filter funnel with the aid of 200 mL of methanol. The pale yellow filtrates from these two solids were combined, 340 mL of ether was added in two portions, and the precipitated white solid was suction filtered, washed with 50 mL of ether and dried over KOH *in vacuo*; 36.6 g of pure product (phosphinic cyclocreatine) were obtained. Anal. Calcd for $C_4H_8N_3O_2P$: C, 29.45; H, 6.18; N, 25.70. Found: C, 29.30; H, 6.05; N, 25.59. HPLC (215 nm detection): 7.2 min.

99.09%. ^{1}HNMR (300 MHz, D_2O) δ 6.95 (dt, 1H, J_{HP} = 528 Hz, J_{HH} = 1.6 Hz), 3.63 (m, 2H), 3.46 (m, 2H), 3.24 (dd, 2H, J_{HP} = 8.5 Hz, J_{HH} = 1.7 Hz).

Example 2- Evaluation of Substrate Activity of A Phosphinic Creatine Compound

Phosphinic cyclocreatine (PhCCr) was evaluated for substrate activity using a coupled assay (see (a) Stauffer Chemical Co.; Brit. 934,090 (Cl. C. 07f); Aug. 14, 1963. (b) Toy, A.D.F.; Rattenbury, K.H.; U.S. Patent 4,244,745; 19 Nov. 1964. (c) Toy, A.D.F.; Ehling, E.H. U.S. Patent 4,160,632; 30 Jan. 1961). K_m,V_{max} and k_{cat} values were graphically determined by the standard manner using double reciprocal plots. Creatine kinase from rabbit muscle (creatine phosphokinase, Sigma, 60 units/mg prot., Cat. No. C-6638) was used for enzyme assays. Kinetic data were collected and processed on a Beckman DU 70 UV/visible spectrophotometer. Assays were run at 37°C with the disappearance of NADH monitored at 340 nm. Assay solutions contained 0.1 M HEPES (pH 7.3), 6 mM $Mg(OAc)_2$, 0.1 M KOAc, 3 M ATP, 0.65 M PEP, 0.39 M NADH, 0.2 mg/mL pyruvate kinase (Sigma), 0.2 mg/mL lactate dehydrogenase (Sigma), and 1.6 μg/mL creatine kinase.

The following values were obtained for PhCCr for the desired kinetic and thermodynamic constants: K_m = 143 mM; V_{max} = 5.6 mmol/min/mg protein; k_{cat} = 455 min^{-1}.

Example 3- *In Vitro* Inhibition of Viral Plaque Formation By A Phosphinic Creatine Compound

Materials and Methods

Viral Strains and Culture

Human cytomegalovirus (HCMV) strain AD169 (ATCC) and herpes simplex virus type 1 (HSV-1) strain F (ATCC) were used for viral plaque reduction assays. MRC-5 human embryonic lung fibroblast cells were purchased from ATCC.

Assays

The cells were grown to confluence in twenty four well plates and infected with a known amount of PFU (Plaque Forming Units; approximately 50 PFU/well) suspended in EMEM JRH Biosciences) containing 2% FBS (Fetal Bovine Serum, JRH Biosciences). The virus was allowed to adsorb for one hour at 37°C, and then media containing a range of concentrations of PhCCr was added to the wells. This was done in triplicate for each concentration of PhCCr. The plates were incubated at 37°C under 5% CO_2 until plaques were identified in the control wells (approximately 7 days post-infection for HCMV and 2 days post-infection for HSV-1). The media was removed from the wells by aspiration, and 400μL of 0.1% crystal violet in 35% methanol was added to fix and stain the cells. The wells were washed with water and dried, and plaques were counted using a dissecting microscope.

PhCCr was examined for its ability to inhibit the formation of viral plaques *in vitro* by HCMV and HSV-1 in MRC-5 cells. The average effective dose, 50% endpoint (ED_{50}), values of PhCCr for HCMV and HSV-1 were 2.3 and 5.8 mM, respectively. The results are graphed in Figures 1-2. This example demonstrates that PhCCr acts as an antiviral agent against both HCMV and HSV-1.

Example 4- A Comparison of The Solubility of A Phosphinic Creatine Compound To Its Non-Phosphinic Creatine Compound Counterpart

The limits of solubility of phosphinic cyclocreatine (PhCCr) and cyclocreatine (CCr) were determined. The solubility of each compound was assessed in phosphate buffered saline (PBS) and two temperatures were selected (room temperature and 4 °C). Both compounds were dissolved in 1X PBS (JRH BioSciences). The stock solution of CCr was made at 20 mg/mL which went into solution by incubating briefly at 65 °C and vortexing. Further dilutions of CCr were done in PBS to give additional final concentrations of 15, 12.5 and 10 mg/mL. The CCr solutions were colorless. PhCCr was made in a concentrated solution of 385 mg/mL, by heating briefly at 65 °C and vortexing. The PhCCr solution was the color of amber. The 385 mg/mL PhCCr precipitated out of solution when incubated briefly on ice and for a longer period at room temperature. The non-chemical particulate fibers present in the 385 mg/mL solution were removed by filtering with a syringe 0.45 um glass filter. Solutions of 300, 250, 200, 150, and 100 mg/mL PhCCr were made by dilution into PBS. One set each of the CCr and PhCCr solutions were placed at room temperature and at 4 °C. The solutions were checked for precipitates at four hours, three days, six days, and nine days after preparation.

Results

After four hours at room temperature and 4 °C, all of the concentrations of CCr (20, 15, 12.5, and 10 mg/mL) and PhCCr (300, 250, 200, 150, and 100 mg/mL) were still in solution. After three days, six days, and nine days, all of the solutions of CCr and PhCCr held at room temperature were still in solution. All of the PhCCr solutions kept at at 4 °C were also still in solution. However, two of the CCr solutions incubated at 4 °C (20 and 15 mg/mL) had particulate in the bottom of the tubes by day three. The 12.5 and 10 mg/mL solutions of CCr were still in solution at 4 °C on day nine. These results demonstrate that PhCCr is at least twenty-four times more soluble than CCr when dissolved in PBS ($\leq$300 mg/mL divided by 12.5 mg/mL).

EQUIVALENTS

Those skilled in the art will recognize or be able to ascertain, using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

CLAIMS

1. A method for treating a subject for viral infection, comprising:
administering an antiviral effective amount of a phosphinic creatine compound to a subject such that the subject is treated for viral infection.

2. The method of claim 1 wherein the subject is treated for viral infection by reducing or eliminating symptoms associated with a pre-existing viral infection.

3. The method of claim 1 wherein the subject is treated for viral infection by preventing the occurrence of viral infection within the subject.

4. The method of claim 1 wherein the virus is a herpes virus.

5. The method of claim 4 wherein the herpes virus is Herpes Simplex Virus Type 1.

6. The method of claim 4 wherein the herpes virus is Herpes Simplex Virus Type 2.

7. The method of claim 4 wherein the herpes virus is a cytomegalovirus.

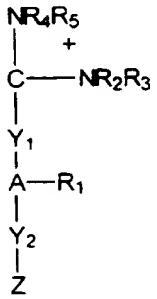
8. The method of claim 7 wherein the cytomegalovirus is human.

9. The method of claim 4 wherein the herpes virus is varicella-zoster virus.

10. The method of claim 1 wherein the virus is a human immunodeficiency virus.

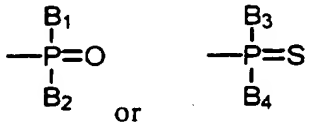
11. The method of claim 1 wherein the virus is an adenovirus.

12. The method of claim 1 wherein the phosphinic creatine compound has a formula as follows:



wherein A is selected from the group consisting of N or CH;

Z is



or

wherein B_1-B_4 are each independently selected from the group of hydrogen and -OX_4 and X_1-X_4 are each independently selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl and pharmaceutically acceptable salts.;

Y_1 and Y_2 are each independently selected from the group consisting of a direct bond, alkylene, alkenylene, alkynylene and alkoxylene;

R_1 is selected from the group consisting of hydrogen, hydroxyl, alkyl, alkenyl, alkynyl, and alkoxyl; and

R_2 - R_5 are each independently selected from the group consisting of hydrogen, a phosphorus containing moiety, alkyl, alkenyl, alkynyl, alkoxy and haloalkyl,

wherein A may form a ring structure with one of the nitrogens in the amidino moiety or with Y_2.

13. The method of claim 12 wherein Z is a phosphinic acid moiety.

14. The method of claim 1 wherein the phosphinic compound is at least ten times more soluble than its corresponding non-phosphinic creatine counterpart.

15. The method of claim 1 wherein the phosphinic creatine compound is phosphinic cyclocreatine.

16. The method of claim 1 wherein the phosphinic creatine compound is phosphinic cyclocreatine phosphate.

17. The method of claim 1 wherein the phosphinic creatine compound is phosphinic creatine.

18. The method of claim 1 wherein the phosphinic creatine compound is phosphinic creatine phosphate.

19. The method of claim 1 wherein the phosphinic creatine compound is phosphinic homocyclocreatine.

20. The method of claim 1 wherein the phosphinic creatine compound is selected from the group of phosphinic creatine compounds set forth in Table 1.

21. The method of claim 12 wherein A forms a ring structure with one of the nitrogens in the amidino moiety of the phosphinic creatine compound.

22. The method of claim 21 wherein the ring structure is a five membered hetero ring structure.

23. The method of claim 12 wherein A forms a ring structure with Y_2 in the phosphinic creatine compound.

24. The method of claim 23 wherein the ring structure is a four-membered hetero ring structure.

25. The method of claim 23 wherein the ring structure is a five-membered hetero ring structure.

26. The method of claim 1 further comprising coadministering a different antiviral agent.

27. The method of claim 26 wherein the antiviral agent is a nucleoside

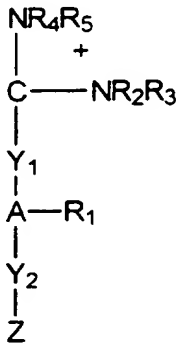
28. The method of claim 27 wherein the nucleoside analog is selected from the group consisting of ganciclovor, idoxoridine, trifluoridine, vidarabine, dideoxyinosine, and azidothymidine.

29. The method of claim 27 wherein the nucleoside acyclovir.

30. The method of claim 26 wherein the antiviral agent is foscarnet.

31. A therapeutic composition for treating a subject for viral infection, comprising
an antiviral effective amount of a phosphinic creatine compound; and
a pharmaceutically acceptable carrier.

32. The therapeutic composition of claim 31 wherein the phosphinic creatine compound has a formula as follows



wherein A is selected from the group consisting of N or CH;

Z is a phosphinic acid moiety;

Y_1 and Y_2 are each independently selected from the group consisting of a direct bond, alkylene, alkenylene, alkynylene and alkoxylene;

R_1 is selected from the group consisting of hydrogen, hydroxyl, alkyl, alkenyl, alkynyl, and alkoxyl; and

R_2 - R_5 are each independently selected from the group consisting of hydrogen, a phosphorus containing moiety, alkyl, alkenyl, alkynyl, alkoxy and haloalkyl,

wherein A may form a ring structure with one of the nitrogens in the amidino moiety or with Y_2.

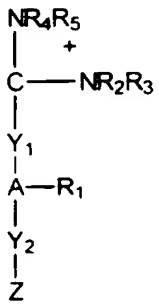
33. The therapeutic composition of claim 31 wherein the creatine compound is phosphinic cyclocreatine.

34. The therapeutic composition of claim 26 further comprising a different antiviral agent.

35. A packaged antiviral agent, comprising

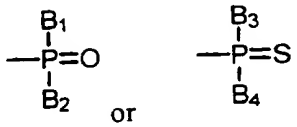
a phosphinic creatine compound packaged with instructions for using the phosphinic creatine compound as an antiviral agent.

36. A phosphinic creatine compound having a formula as follows:



wherein A is selected from the group consisting of N or CH;

Z is



or

wherein B_1-B_4 are each independently selected from the group of hydrogen and -OX_4 and X_1-X_4 are each independently selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl and pharmaceutically acceptable salts.;

Y_1 and Y_2 are each independently selected from the group consisting of a direct bond, alkylene, alkenylene, alkynylene and alkoxylene;

R_1 is selected from the group consisting of hydrogen, hydroxyl, alkyl, alkenyl, alkynyl, and alkoxyl; and

R_2 - R_5 are each independently selected from the group consisting of hydrogen, a phosphorus containing moiety, alkyl, alkenyl, alkynyl, alkoxy and haloalkyl,

wherein A may form a ring structure with one of the nitrogens in the amidino moiety or with Y_2, provided that the phosphinic creatine compound is not phosphinic creatine.

37. The phosphinic compound of claim 36 which is selected from those compounds listed in Table 1 provided that the phosphinic creatine compound is not phosphinic creatine.

38. The therapeutic composition of claim 36 wherein the phosphinic creatine compound is phosphinic cyclocreatine.

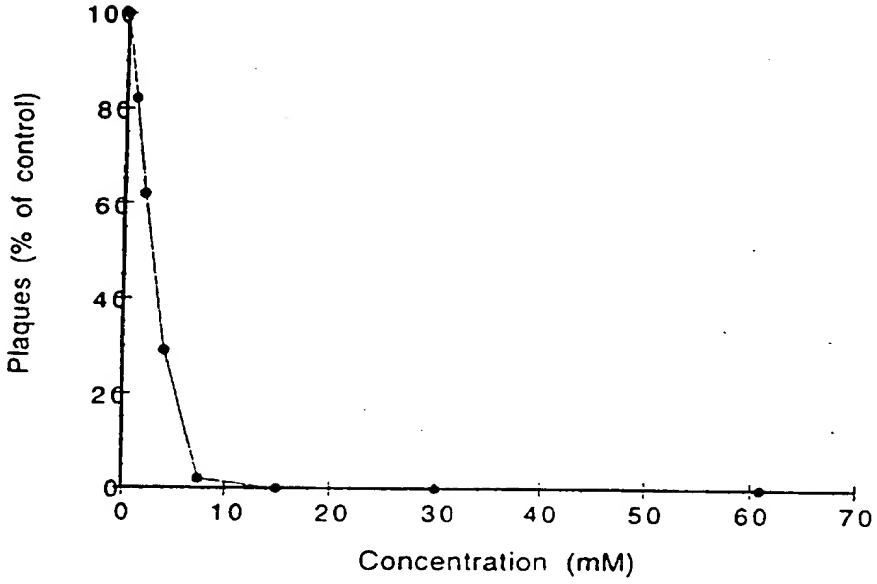

Plaques (% of control)
100
80
60
40
20
0
0
10
20
30
40
50
60
70
Concentration (mM)

FIGURE 1

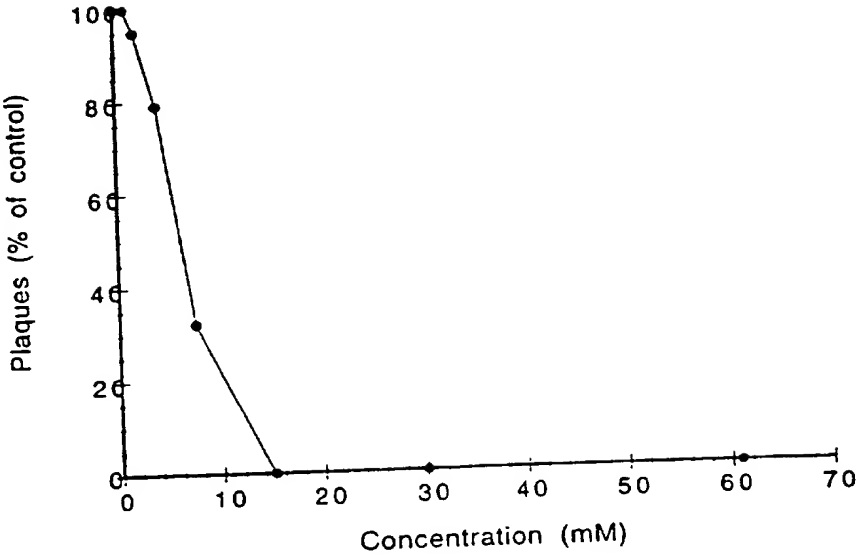

Plaques (% of control)
100
80
60
40
20
0
0
10
20
30
40
50
60
70
Concentration (mM)

FIGURE 2